PRODUCT INFORMATION AND MANUAL

Enzyme-linked immunosorbent assay for quantitative detection of human IL-8

For Research Use Only

Not for diagnostic or therapeutic procedures.

96 Wells

Human IL-8

Product # 451210

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GENERAL INFORMATION

Product Description

The *Human IL-8 ELISA Test Kit* is designed for quantitative determination of the concentration of human interleukin 8 (IL-8) in serum, plasma, and cell culture supernatant.

Human IL-8 is an 8 kD peptide consisting of 77 amino acid residues after cleavage of 22 residue signal peptide. Further proteolysis of the N-terminal end generates a 72 amino acid peptide with full biological activity. As a member of the neutrophil-specific CXC subfamily of chemokines, IL-8 is a potent neutrophil chemtactic and activating factor. In response to proinflammatory stimuli such as IL-1, TNF, LPS and virus, IL-8 is produced by monocytes/macrophages, T cells, neutrophils, fibroblasts, endothelial cells, keratinocytes, hepatocytes, astrocytes and chondrocytes. The function of IL-8 is to attract neutrophils to the site of inflammation and to activate them. IL-8 binds to two G protein-coupled receptors, CXCR1 and CXCR2, as well as to the Duffy antigen on red blood cells.

The specifications of the *Human IL-8 ELISA Test Kit* are as follows:

Format: 96-well strip plate
Assay range: 16-1024 pg/mL
Sensitivity: 16 pg/mL
Total Assay Time: 3 hours and 45 minutes
Sample Size: 100 µL/well
Sample types: Serum, plasma and Cell Culture Supernatant
Wavelength for plate reading: A450 nm

Procedure Overview

The method is a solid phase sandwich ELISA (Enzyme-Linked Immunosorbent Assay). It utilizes a monoclonal antibody (capture antibody) specific for human IL-8 coated on a 96-well plate. Standards and samples are added to the wells, and any human IL-8 present binds to the immobilized antibody. The wells are washed and biotinylated polyclonal anti-human IL-8 antibody (detection antibody) is added. After a second wash, avidin-horseradish peroxidase (avidin-HRP) is added, producing an antibody-antigen-antibody sandwich. The wells are again washed and a substrate solution is added, which produces a blue color in direct proportion to the amount of human IL-8 present in the initial sample. The stop buffer is then added to terminate the reaction. This results in a color change from blue to yellow. The wells are then read at 450 nm.

Kit Contents, Storage and Shelf Life

The *Human IL-8 ELISA Test Kit* has the capacity for 96 determinations or testing of 40 samples in duplicate (assuming 16 wells for standards and negative controls). Return any unused microwells to the foil bag and reseal them with the desiccant provided in the original package. Store the kit components as recommended in the table below. The shelf life is 12 months when the kit is properly stored.

Kit Contents	Amount	Storage
Capture Ab-coated Microtiter Plate	1 x 96-well plate (8 wells x 12 strips)	2-8°C
Human IL-8 Standard	3 x 20 µL	-20°C
250X Detection Antibody	75 µL	-20°C
250X Avidin-HRP	75 µL	-20°C
5X Assay Diluent**	15 mL	2-8°C
20X Wash Solution**	28 mL	2-8°C
Stop Buffer**	20 mL	2-8°C
TMB Substrate**	12 mL	2-8°C

** Components with the same part numbers are interchangeable among kits as long as they are used before their expiration dates.

Required Materials Not Provided With the Kit

- Microtiter plate reader (*450 nm*)
- 10, 20, 100 and 1000 µL pipettes
- Multi-channel pipette: 50-300 µL (*Optional*)
- Distilled deionized water

Sensitivity (Detection Limit)

16 pg/mL

Specificity (Cross-Reactivity)

Analytes	Cross-Reactivity (%)
Human IL-8:	100
Human GM-CSF:	<0.01
Human IL-1:	<0.01
Human IL-4:	<0.01
Human TGF-β:	<0.01
Human IL-5:	<0.01
Mouse GM-CSF:	<0.01
Mouse IL-1:	<0.01
Mouse IL-4:	<0.01
Mouse TGF−β:	<0.01
Mouse IL-6:	<0.01
Mouse IL-15	<0.01
Mouse IFN-γ:	<0.01
Mouse IL-17:	<0.01
Rat IL-6:	<0.01
Rat TNF-α:	<0.01

Warnings and Precautions

Neogen strongly recommends that you read the following warnings and precautions to ensure your full awareness of ELISA techniques and other details you should pay close attention to when running the assays. More information can also be found in the Troubleshooting section. Periodically, optimizations and revisions are made to the kit and manual. Therefore, it is important to follow the version of the protocol included with the kit. If you need further assistance, please contact a Neogen technical services representative at techservice-lifesciences@neogen.com.

- The standard contains human IL-8. Handle with particular care.
- Do not use the kit past the expiration date.
- Do not intermix reagents from different kits or different lots. ANTIBODIES AND PLATES ARE KIT- AND LOT-SPECIFIC. Make sure that the standards, detection antibody, avidin-HRP, and diluent are mixed in correct volumes.
- Try to maintain a laboratory temperature of (20 – 25°C / 68 – 77°F). Avoid running assays under or near air vents, as this may cause excessive cooling, heating and/or evaporation. Also, do not run assays in direct sunlight, as this may cause excessive heat and evaporation. Cold bench tops should be avoided by placing several layers of paper towel or some other insulation material under the assay plates during incubation.
- Make sure you are using only distilled deionized water since water quality is very important.
- Incubations of assay plates should be timed as precisely as possible. Be consistent when adding standards to the assay plate. Add your standards first and then your samples.
- Add standards to plate only in the order from low concentration to high concentration as this will minimize the risk of compromising the standard curve.
- Always refrigerate plates in sealed bags with a desiccant to maintain stability. Prevent condensation from forming on plates by allowing them to equilibrate to room temperature (20 – 25°C / 68 – 77°F) before opening (plates provided in packaging contain dessicant).
- Be sure samples are properly stored. In general, samples should be refrigerated at 2-4°C for no more than 1-2 days. Freeze samples to -20°C or colder if they need to be stored for a longer period. Frozen samples can be thawed at room temperature (20 – 25°C / 68 – 77°F) or in a refrigerator before use.
- Human IL-8 in plasma, sera, and cell culture supernatant can be measured directly using this kit without extraction.

HUMAN IL-8 ELISA TEST KIT PROTOCOL

Reagent Preparation

IMPORTANT: All reagents should be brought up to room temperature before use (1 – 2 hours at 20 – 25°C / 68 – 77°F). Make sure you read the "Warnings and Precautions" section on page 3. Solutions should be prepared just prior to ELISA test. All reagents should be mixed by gently inverting or swirling prior to use. Prepare volumes that are needed for the number of wells being run. Do not return the reagents to the original stock tubes/bottles. Disposable reservoirs are recommended to minimize the risk of contamination.

1. **Preparation of 1X Assay Diluent**
   Mix 1 volume of 5X Assay Diluent with 4 volumes of distilled water. In general, 50 mL of 1X Assay Diluent is sufficient for one whole plate.
2. **Preparation of Standards**
   Add 10 µL of Standard solution to 1 mL of 1X Assay Diluent to prepare 1024 pg/mL. Make series 2X dilutions in the same diluent to make standards of 512 pg/mL, 256 pg/mL, 128 pg/mL, 64 pg/mL, 32 pg/mL, and 16 pg/mL. Use 1X Assay Diluent as negative control.
3. **Preparation of 1X Wash Solution**
   Mix 1 volume of the 20X Wash Solution with 19 volumes of distilled water.
4. **Preparation of 1X Detection Antibody**
   Mix 1 volume of 250X Detection Antibody with 249 volumes of 1X Assay Diluent.
5. **Preparation of 1X Avidin-HRP**
   Mix 1 volume of 500X Avidin-HRP with 249 volumes of 1X Assay Diluent.

ELISA Testing Protocol

Label the individual strips that will be used and prepare working solutions of reagents as shown in the following example. Adjust the total amount as needed for number of wells that will be used.

Component	Volume per Well	24 Wells
1X Detection Antibody	100 µL	2.4 mL
1X Avidin-HRP	100 µL	2.4 mL
1X Wash Solution	3.0 mL	72 mL
Stop Buffer	100 µL	2.4 mL
TMB Substrate	100 µL	2.4 mL

1. Add 100 µL of 1X Assay Diluent to duplicate wells to serve as negative controls. Add 100 µL of each dilution of Human IL-8 Standards in duplicate into different wells (✐ *Add standards to plate only in the order from low concentration to high concentration*).
2. Add 100 µL of each sample in duplicate into different sample wells.
3. Incubate for 2 hours at room temperature (*20 – 25°C / 68 – 77°F*) (✐ *Avoid direct sunlight and cold bench tops during the incubation. Covering the plate with aluminum foil while incubating is recommended*).
4. Aspirate all fluid from each well after the incubation and wash wells 3 times with 250 µL of 1X Wash Solution per wash. It is not necessary to agitate the plate during the wash steps. Allow Wash Solution to remain in wells for 1-2 min during each wash step. After last wash,

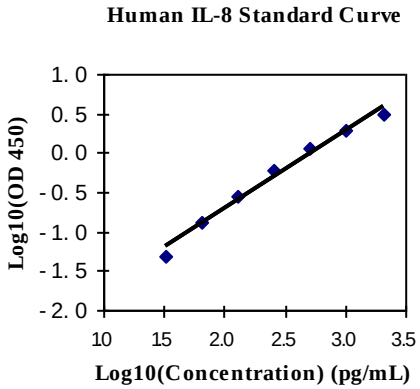
invert the plate and gently tap the plate on paper towels to remove residual fluid. (✍ *Perform the next step immediately after plate washings. Do not allow the plate to air dry between working steps*).

5. Add 100 µL of 1X Detection Antibody into each well and incubate the plate for 1 hour at room temperature.
6. Aspirate and wash the plate 3 times with 250 µL of 1X Wash Solution per wash. After the last wash, invert the plate and gently tap the plate on paper towels (✍ *Perform the next step immediately after the third wash. Do not allow the plate to air dry between steps*).
7. Add 100 µL of 1X Avidin-HRP into each well. Incubate the plate for 30 minutes at room temperature (*20 – 25°C / 68 – 77°F*).
8. Wash the plate 3 times with 250 µL of 1X Wash Solution per wash. After the last wash, invert the plate and gently tap the plate dry on paper towels (✍ *Perform the next step immediately after the third wash. Do not allow the plate to air dry between steps.*)
9. Add 100 µL of TMB Substrate and incubate for 15 minutes at room temperature (*20 – 25°C / 68 – 77°F*). Start timing the reaction immediately after adding the substrate to the last well. Mix the solution by gently rocking the plate manually during the first minute of incubation. (🛇 ✍ *Do not put any substrate back into the original container which could lead to contamination. Any substrate solution exhibiting coloration is indicative of deterioration and should be discarded. Covering the plate with aluminum foil while incubating is recommended*).
10. Add 100 µL of Stop Buffer in the order of adding TMB substrate to stop the enzyme reaction.
11. Read the plate as soon as possible following the addition of Stop Buffer. Read plate on a plate reader at 450 nm wavelength (✍ *Before reading, wipe the bottom of the plate with a lint-free tissue to remove any moisture or fingerprints that could interfere with the reading.*)

Human IL-8 Concentration Calculations

A standard curve can be constructed by plotting the adjusted average absorbance for each reference standard against its concentration in pg/mL on a logarithmic scale as shown in the figure below. The adjusted average absorbance is obtained by subtracting the average of the negative control absorbance from the average of each observed absorbance.

To determine the corresponding concentration of human IL-8 in pg/mL in samples, plot the adjusted absorbance value for each sample on the standard curve. The figure to the right shows a typical human IL-8 standard curve.



TROUBLESHOOTING

No Color Development or No Signals with Standards

Possible Causes	Recommended Action
Reagents were used in the wrong order or a step was skipped.	Follow the protocol carefully and repeat the assay.
Wrong antibodies were used. Either Detection Antibody or Avidin-HRP was prepared incorrectly or has deteriorated.	Make sure that the antibodies used are the ones that came with the kit. All antibodies are kit- and lot-specific. Make sure that the Detection Antibody, Avidin-HRP and diluent are mixed in correct volumes.
TMB Substrate has deteriorated.	Use a new set of TMB substrate. Note, if TMB substrate shows any color before use, it should not be used for the assay.

Low Optical Density (OD) Readings

Possible Causes	Recommended Action
Reagents were expired or mixed with a different lot number.	Verify the expiration dates and lot numbers.
Wash solution was prepared incorrectly.	Verify that the solution was prepared as described in the protocol.
Too many wash cycles were used.	Make sure to use the number of washes per the protocol instruction.
Incubation times were too short.	Follow protocol and ensure accurate incubation time.
Lab temperature was too low.	Maintain the lab room temperature within (20 – 25°C / 68 – 77°F). Do not run assays under air conditioning vents or near cold windows.
Reagents and plates were too cold.	Make sure that the plates and reagents are brought up to room temperature.
Reader was at wrong wavelength, or reader was malfunctioning.	Make sure that the wavelength is set to 450 nm and read the plate again. Verify reader calibration and lamp alignment.
Excessive kit stress has occurred.	Check records to see how many times the kit has cycled from the refrigerator. Check to see if the kit was left at extreme temperatures for too long.
Assay plates were compromised.	Always refrigerate plates in sealed bags with a desiccant to maintain stability. Prevent condensation from forming on plates by allowing them to equilibrate to room temperature (20 – 25°C / 68 – 77°F) while in the packaging.

High Background or High Optical Density (OD) Readings

Possible Causes	Recommended Action
Poor quality water was used in wash solution.	If water quality is questionable, try substituting an alternate source of distilled deionized water to prepare the wash solution.
Substrate solution has deteriorated.	Make sure that the substrate is colorless prior to addition to the plate.
There was insufficient washing or poor liquid handling technique.	Use the number of washes per the protocol instruction. Make sure that 250 µL of wash solution is dispensed per well per wash. If you use a multichannel pipette or robotic liquid handling system, verify its performance; have the system repaired if any ports drip, dispense or aspirate poorly.
Reader was malfunctioning or not blanked properly. This is a high possibility if the OD readings were high and the color was light.	Verify the reader's performance using a calibration plate and check the lamp alignment. Verify the blanking procedure, if applicable, and reblank.
Lab temperature was too high.	Maintain the room temperature within (20 – 25°C / 68 – 77°F). Avoid running assays near heat sources or in direct sunlight.
Reagents were intermixed, contaminated or prepared incorrectly.	Ensure that the correct reagents were used, that working solutions were prepared correctly and that contamination has not occurred.

High Intra-Plate or Inter-Plate Variance

Possible Causes	Recommended Action
Inconsistent time was taken when adding standards, reagents or samples within and/or between plates.	Make sure that all materials are set up and ready to use. Use a multichannel pipette to add reagents to multiple wells whenever possible. Do not interrupt procedure while adding standards, reagents and samples.
Multichannel pipette was not functioning properly.	Verify pipette calibration and check that tips are on tight. Be sure all channels of the pipette draw and dispense equal volumes.
There was inconsistent washing or poor liquid handling technique.	Use the number of washes per the protocol instruction. Make sure that 250 µL of wash solution is dispensed per well per wash. If you use a multichannel pipette or robotic liquid handling system, verify its performance; have the system repaired if any ports drip, dispense or aspirate poorly.
Inconsistent incubation times occurred from plate to plate.	Time each plate separately to ensure consistent incubation times.
Pipette was inaccurate.	Check the pipette calibration. Verify that pipette tips are on tight before use and that all channels draw and dispense equal volumes.
Kit plates, reagents, standards and samples were at different temperatures.	Make sure to allow sufficient time for kit plates, reagents, standards and samples to come to room temperature (20 – 25°C / 68 – 77°F). Larger volumes will require longer equilibration time. If using a water bath to hasten equilibration, make sure that it is maintained at room temperature; do not use a warm water bath to warm reagents, samples and kit standards.
Reagents used were intermixed from different kit lots, or the kits were of different expiration dates.	Carefully label each user-prepared reagent to make sure that the reagents are not intermixed. Kits with different expiration dates might generate different range of OD readings, however, the relative absorbance values will typically be comparable. In general, a value of less than 1.0 reading for the highest standard may indicate deterioration of reagents.

One or More of the Standard Curve Points Are Out of Range

Possible Causes	Recommended Action
Standards were added in wrong order or recorded in wrong position.	Make sure that the standards are applied and recorded correctly.
Standards were contaminated or intermixed with other standards.	Prepare a new set of standards. Always add standards to plate in the order from low concentration to high concentration.
There was inconsistent washing or poor liquid handling technique.	Use the number of washes per the protocol instruction. Make sure that 250 µL of wash solution is dispensed per well per wash. If you use a multichannel pipette or robotic liquid handling system, verify its performance; have the system repaired if any ports drip, dispense or aspirate poorly.
Inconsistent time was taken to add standards and reagents to plate.	Make sure all materials are set up and ready to use. Add standards to plate only in the order from low concentration to high concentration at undisrupted constant pace. Use a multichannel pipette to add reagents to multiple wells simultaneously to increase consistency.
Multichannel pipette was not functioning properly.	Verify pipette calibration and check that tips are on tight. Be sure all channels of the pipette draw and dispense equal volumes.

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